GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

No action as of 8/6

Date: June 18, 1976

Project Title: pH Effect upon "Critical"ATP Level in Heart Muscle

Project No: G-32-631

Project Director: Dr. Gary L. Anderson

Sponsor: Georgia Heart Association

Agreement Period: From 7/1/76 Until 6/30/77

Type Agreement: Letter dated 6/4/76

Amount: $10,738

Reports Required: Annual (Final) Progress Report; Publication Reprints

Sponsor Contact Person (s):

Technical Matters

C. Daniel Cabaniss, M.D., President
GEORGIA HEART ASSOCIATION, INC.
Broadview Plaza, Level C
2581 Piedmont Road, NE
Atlanta, Georgia 30324

Contractual Matters
(thru OCA)

Defense Priority Rating:

Assigned to: Biology (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director—EES
- Accounting Office
- Procurement Office
- Security Coordinator (OCA) ✓
- Reports Coordinator (OCA)
- Library, Technical Reports Section
- Office of Computing Services
- Director, Physical Plant
- EES Information Office
- Project File (OCA)
- Project Code (GTRI)
- Other

CA—3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

no action

Date: 4/11/78

Project Title: pH Effect upon "Critical" ATP Level in Heart Muscle

Project No: G-32-631

Project Director: G.L. Anderson

Sponsor: Georgia Heart Association

Effective Termination Date: 6/30/77 (Grant Expiration)

Clearance of Accounting Charges: n/a - all have cleared

Grant/Contract Closeout Actions Remaining: none

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
_ Other _____

Assigned to: Biology (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director—EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)
Library, Technical Reports Section
Office of Computing Services
Director, Physical Plant
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other _____

CA—4 (3/76)

G-32-631

RESEARCH REPORT FORM

(for Georgia Heart Association Reports ONLY)

(Please type)

NAME OF INVESTIGATOR : Gary L. Anderson, Ph.D.

INSTITUTIONAL ADDRESS : Georgia Institute of Technology
School of Biology
Atlanta, Georgia 30332

TITLE OF RESEARCH PROJECT : Effect Upon "Critical ATP Level in Heart Muscle"

PERIOD COVERED BY THIS REPORT : June, 1976 - June, 1977

TECHNICAL RESEARCH REPORT

Objectives: The ultimate objective of this study is to evaluate the influence of pH upon the relationship between mechanical performance and ATP concentration in mammalian cardiac muscle. In order to achieve this objective mechanical performance and ATP concentration must be accurately measured. In addition a measurement of tissue mass and calculation of cross-sectional area are required in order to normalize ATP content and mechanical performance for samples of varying size. This report details progress which has been made in perfecting these measurements for small sample sizes.

Mechanical performance: The preparation used in this study is the guinea pig papillary muscle. The techniques employed to quantify mechanical performance are similar to those described in the literature for mammalian papillary muscle preparations. Peak isometric tension is measured and normalized to muscle cross sectional area. In order to evaluate the influence of pH on critical ATP level, a procedure for creating an imbalance between energy supply and demand has been

developed. It is accomplished by exposing isolated papillary muscles to experimental anoxia. Details for the experimental set up used in this procedure are given in the accompanying Appendix. Typical responses of preparations exposed to experimental anoxia are shown in Figures 1 and 2. The effect of pH on this response can be evaluated changing the pH at the onset of the anoxic exposure; 7.1 to 7.6 as in Figure 1 or 7.4 to 7.6 as in Figure 2. The results of these preliminary experiments show that changes in peak developed tension can be used to assess mechanical effects of experimental anoxia in these preparations. In addition it appears that the response during experimental anoxia is dependent upon the pH of the bathing media. At pH 7.6 developed tension decreases less rapidly than at either pH 7.1 or 7.4. Experiments will be done to determine the ATP content of muscles exposed to experimental anoxia at 6 min. into the anoxic period. It is at this point that the maximum differences in developed tension between muscles bathed at different pHs is observed, see Figures 1 & 2. In addition ATP content at the point developed tension is reduced to 50% of that in the control period will be determined.

Measurements have also been made of ATP content of muscles exposed to experimental anoxia. The details of this measurement are described elsewhere. After 10 min. of experimental anoxia muscles maintained at pH 7.1 have a mean ATP concentration of 40 $\pm$ 3.7 percent of that at the end of the equilibration period. These observations establish the feasibility of measuring ATP concentration at a predetermined point during experimental anoxia. Experiments are now underway to determine ATP concentration in papillary muscle in which peak tension has been reduced to 50% of that at the equilibration period.

ATP content: A significant part of our effort to date has been to develop

FIGURE 1

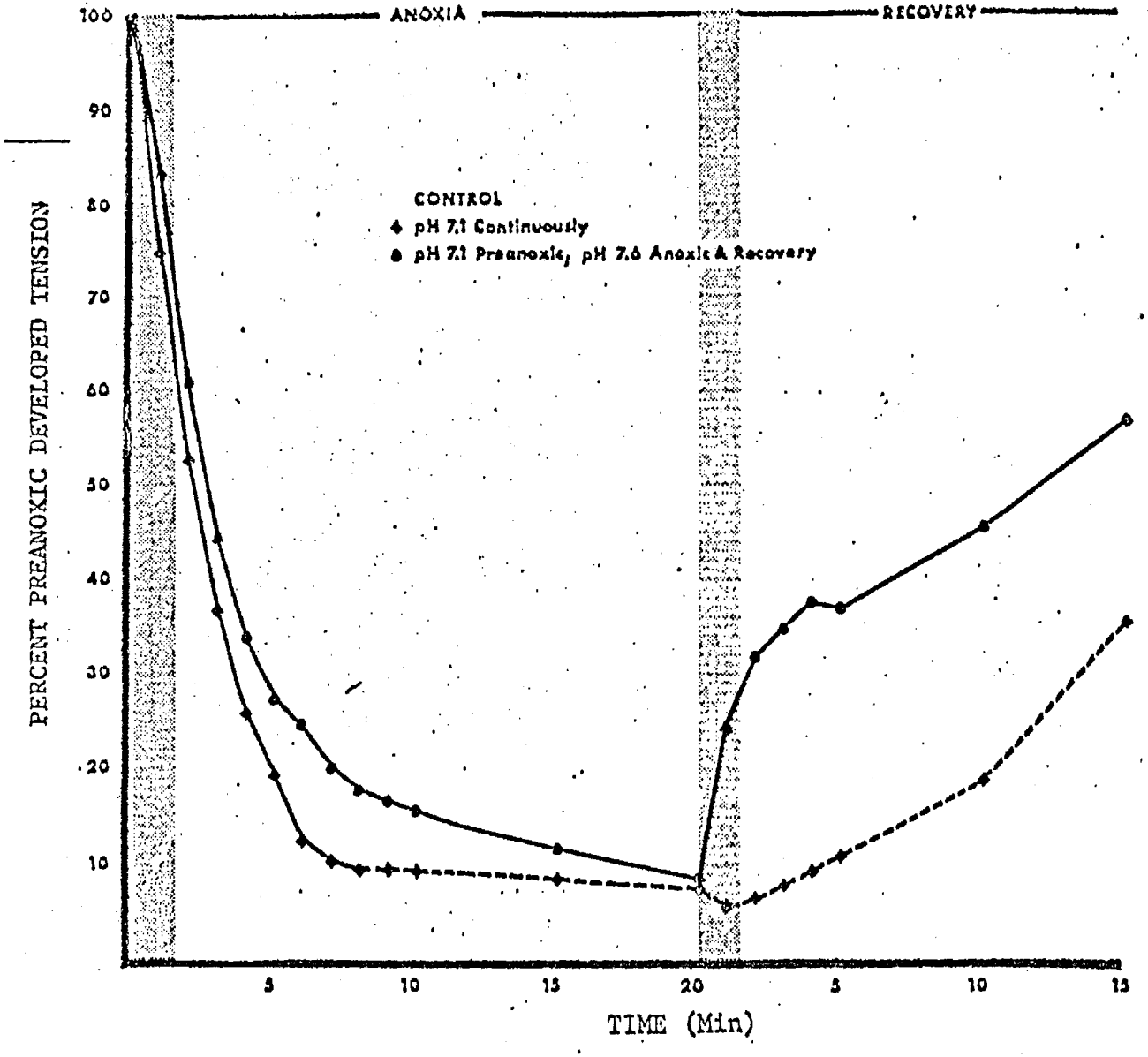
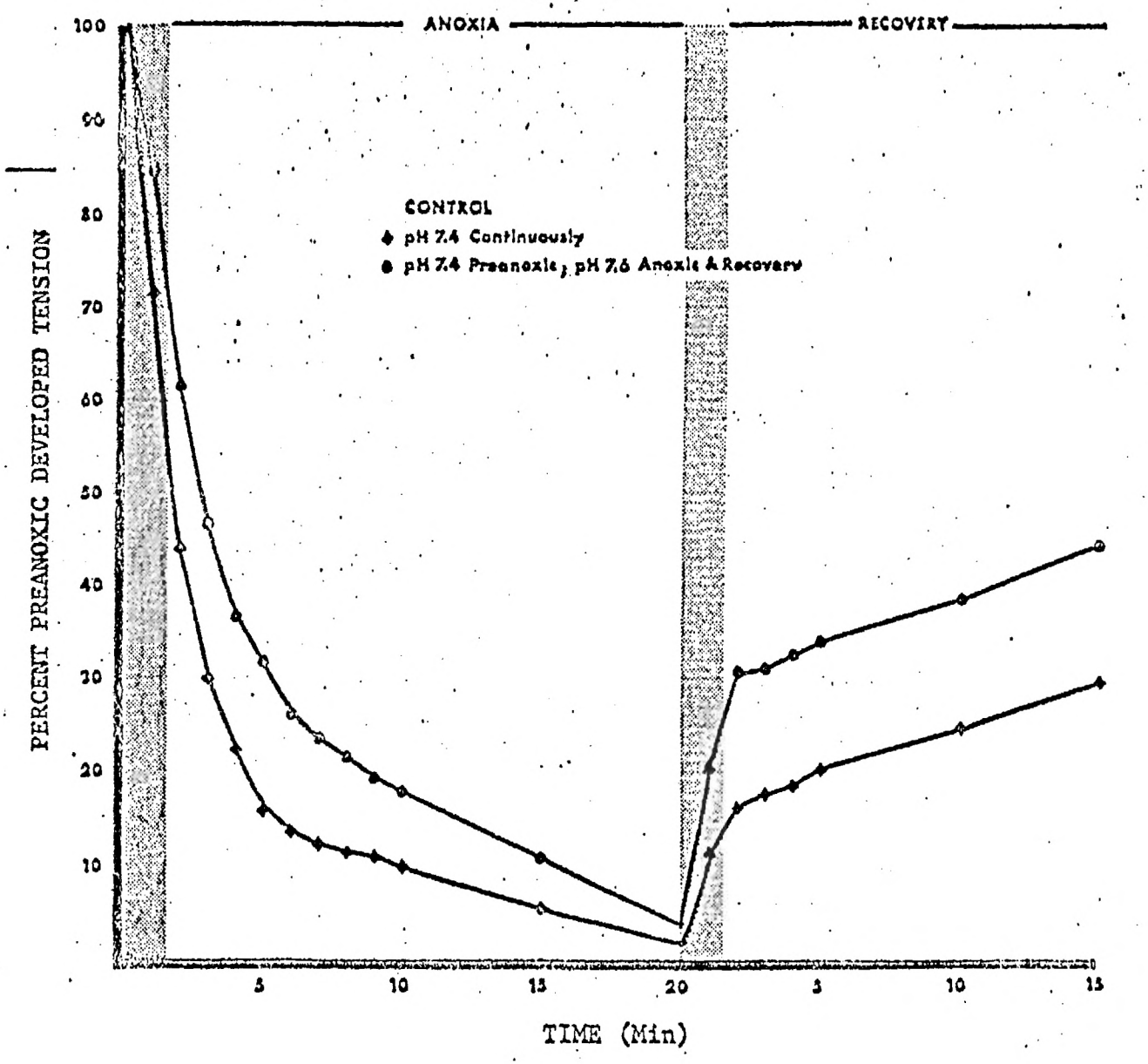

ANOXIA
RECOVERY
CONTROL
pH 7.1 Continuously
pH 7.1 Preanoxic, pH 7.6 Anoxic & Recovery
PERCENT PREANOXIC DEVELOPED TENSION
100
90
80
70
60
50
40
30
20
10
5
10
15
20
5
10
15
TIME (Min)

FIGURE 2


ANOXIA
RECOVERY
CONTROL
pH 7.4 Continuously
pH 7.4 Preanoxia, pH 7.6 Anoxia & Recovery
PERCENT PREANOXIC DEVELOPED TENSION
100
90
80
70
60
50
40
30
20
10
5
10
15
20
5
10
15
TIME (Min)

techniques which permit quantification of ATP in small samples. The process involves two steps (1) the extraction of ATP from the tissue and (2) the quantification of ATP concentration in the extract. Quantification of ATP in extracts is done using a modification of the luciferin-luciferase reaction. The required sensitivity is achieved by using the Luminescence Biometer Reagent Kit (DuPont Inst.) and making measurements on a Chem-Glow Photometer (Aminco).

Several techniques for extraction of ATP in perchloric acid have been evaluated. These include extraction of ATP from frozen powdered tissue, extraction from thin (8 μ) frozen sections of muscle, and extraction from freeze dried muscle. The latter has proven most reproducable and has the added advantage that the freeze dried papillary muscle can be weighed at room temperature without hydrolysis of ATP. The results of ATP determinations done on ventricular muscle extracted in this manner are shown in Table 1. Each sample represents a portion of a large piece of guinea pig ventricular muscle which was frozen in aluminum tongs cooled to the temperature of liquid nitrogen. This section is broken into smaller samples which are freeze dried under identical conditions. The dried samples are weighed on an electrobalance (Cahn Model M10) and sonicated in 1 ml of .3 M perchloric acid. Samples are allowed to extract for 1 1/2 hours in an ice bath and then centrifuged at 10,000 g for 10 min. Assuming homogeniety of ATP concentration within the ventricle, differences in ATP concentration between samples are related to variability in the techniques for extraction and quantification. The variability in ATP content determined in this manner (i.e., standard deviation of less than 10% of the mean) is less than that determined for extracts from either powdered tissue or thin frozen sections.

TABLE 1

ATP content of guinea pig ventricular muscle

Sample	dry wt(mg)[a]	wet wt(mg)[b]	μ moles ATP/g dry weight
1	3.99	20.1	14.5
2	3.46	17.4	17.2
3	1.46	7.3	15.8
4	2.25	11.3	16.4
5	1.41	7.1	16.7
6	2.50	12.6	13.6
7	1.88	9.4	15.5
8	1.01	5.1	16.9
9	0.69	3.5	17.1
10	2.41	12.1	16.4
11	2.30	11.6	15.6
12	0.85	4.3	14.9
13	1.94	9.7	17.0
14	1.58	7.9	16.3
15	2.14	10.8	17.3
16	1.09	5.5	14.9
			mean 16.0 ± 1.1 S.D.

[a] Dry weight is determined on Cahn Electrobalance (model M10) after 12 hrs. freeze drying of each sample.

[b] Wet weight is calculated using an experimentally determined ratio of wet weight: dry weight for guinea pig ventricular muscle.

Sample size: Determination of sample size is required (1) to normalize ATP content for variations in size of papillary muscles and (2) to permit calculation of cross-sectional area for normalization of mechanical performance. Three alternatives for determination of sample size have been explored. They include weighing of frozen papillary muscles, determination of Lowry protein in perchloric acid extracts, and weighing of freeze dried muscle. The latter has proven to be the most reliable and convenient. One limitation of this approach is that calculation of cross-sectional area requires either a calculated or measured wet weight. The problem has been solved by quantitating the wet weight: dry weight ratio for guinea pig papillary muscle. The results of these determinations are given in Table 2. Duplicate measurements of blotted wet weight were more variable than those of dry weight. We conclude that dry weight is an appropriate parameter with which to quantify sample size and can be used to calculate cross-sectional area.

Summary: Procedures have ~~not~~ been developed which permit quantification of mechanical and chemical parameters necessary to determine critical ATP content in guinea pig papillary muscles. Specific accomplishments include: development of procedures to impose experimental anoxia without mechanical artifact, and to quantitate ATP in freeze dried papillary muscle.

TABLE 2.

Wet weight, dry weight and wet weight: dry weight ratio for guinea pig papillary muscle.

Sample	Wet Weight(mg)[a]	Dry Weight(mg)[b]	$\frac{\text{Wet weight}}{\text{Dry weight}}$
1	4.07	.80	5.10
2	2.50	.51	4.09
3	3.77	.75	5.06
4	9.33	1.81	5.15
5	3.39	.69	4.88
6	5.62	1.24	5.00
7	7.77	1.54	5.03
8	6.34	1.27	4.99
9	4.34	.86	5.05
10	2.95	.70	4.21
11	5.10	.89	5.70
12	5.36	1.01	5.30
13	3.76	.75	5.02
14	7.95	1.58	5.03
			mean 5.03 $\pm$ .31

[a] Wet weight is determined by weighing blotted samples of guinea pig ventricle on a Cahn Electrobalance (model M10).

[b] Dry weight is determined after samples are freeze dried over night, approximately 14 hrs.

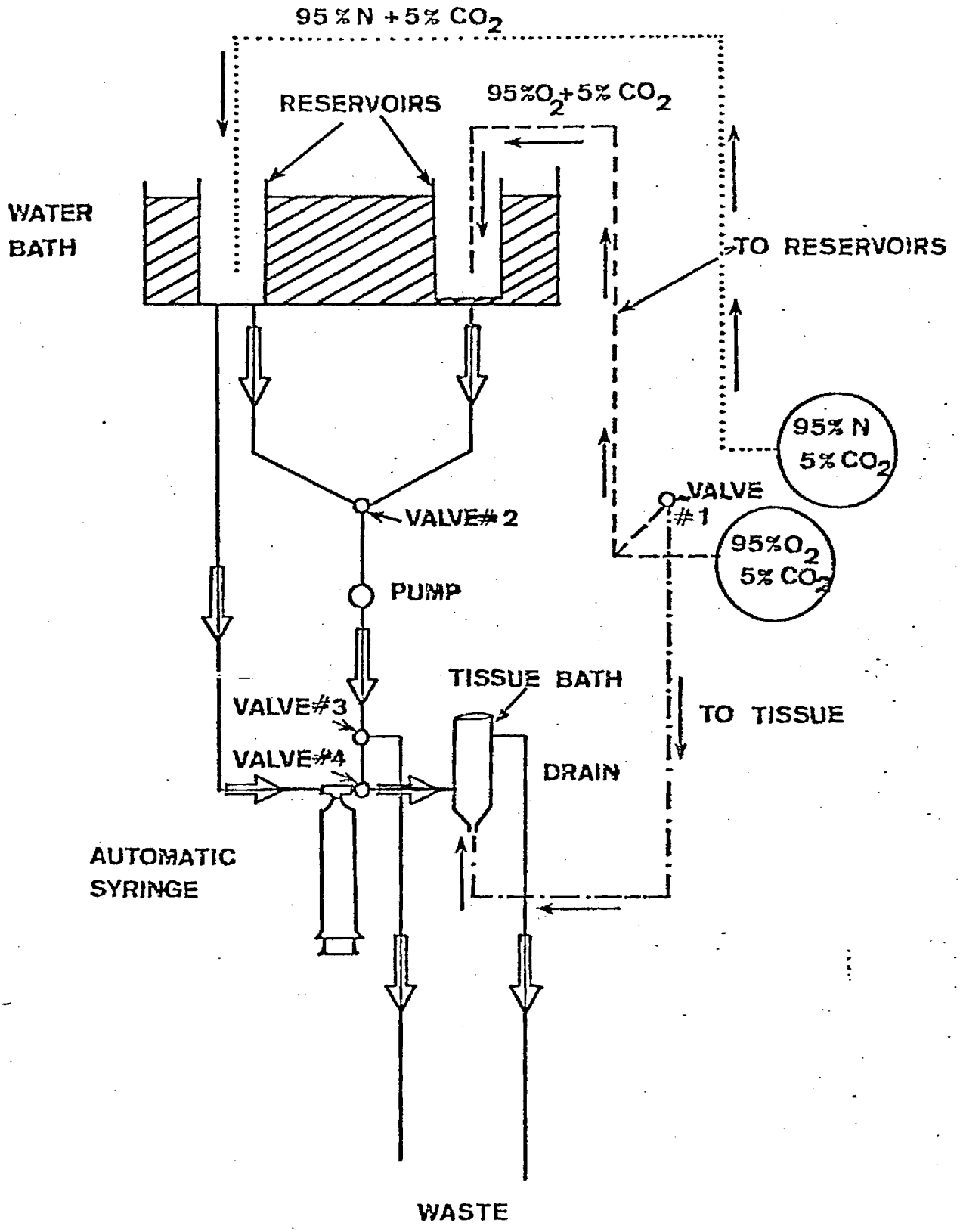
APPENDIX

Section I. Experimental anoxia

The system used for aeration and perfusion of the tissue bath is illustrated in Figure 1. This system permits direct aeration of fluid in the tissue bath as well as continuous perfusion of the bath from an aerated reservoir. In addition experimental anoxia can be induced rapidly without mechanical artifact.

Papillary muscle preparations are equilibrated for a 30-45 min. period prior to being subjected to experimental anoxia. During this period the tissue bath is aerated directly with a 95% O_2 - 5% CO_2 gas mixture (value #1 in the aeration line is set so that the line to the tissue bath carries aerobic gas mixture). At this time the bath is perfused from a temperature controlled reservoir which is aerated with the 95% O_2 - 5% CO_2 mixture (value #2 in the fluid line is set so bathing solution is pumped from the aerobic reservoir and valves #3 and #4 direct the fluid to the tissue bath). Fluid drains by gravity through the drain line into a waste receptacle; there is no recycling of the perfusion fluid.

Experimental anoxia is induced by changing the fluid within the tissue bath, the direct aeration of the tissue bath and the perfusion of the tissue bath. The sequence of steps included: (i) Valve #3 is set so perfusion fluid bypasses the bath and goes directly to waste. (ii) Valve #2 is set so fluid from the reservoir aerated with 95% N_2 - 5% CO_2 fills the fluid lines but temporarily bypasses the tissue bath. At this time the tissue is still aerated with 95% O_2 - 5% CO_2 but is not perfused. Controls were run to show that this is not associated with any deterioration of mechanical performance over even relatively


95% N + 5% CO2
RESERVOIRS
95%O2+5% CO2
WATER BATH
TO RESERVOIRS
95% N 5% CO2
VALVE #1
95%O2 5% CO2
VALVE#2
PUMP
TISSUE BATH
TO TISSUE
VALVE#3
VALVE#4
DRAIN
AUTOMATIC SYRINGE
WASTE

long time periods. (iii) The valve in the gas line is switched to change direct aeration to the anaerobic mixture. (iv) Fifty ml of fluid from the anaerobic reservoir are rapidly flushed through the tissue bath, using a 50 ml autosyringe (Figure 1). Steps (iii) and (iv) can both be completed within a 30 sec. time interval and bring about a rapid change in the PO_2, greater than 90% complete in 1.5 min., in the tissue bath without introducing artifacts into the tension measurements. Step (iii) is taken as the onset of the anoxic period. On the completion of step (iv), valve #3 is turned to permit perfusion of the bath with fresh solution equilibrated with 95% N_2 + 5% CO_2.

Section II. ATP content

Papillary muscles are rapidly frozen with aluminum tongs cooled to the temperature of liquid nitrogen and stored in liquid nitrogen until extracted. Frozen muscles are freeze dried; care is taken to prevent thawing of the tissue during this process. Freeze dried muscles are disrupted by sonication in 1 ml .3 M perchloric acid and extracted for 1/2 hour. Extracted samples are centrifuged at 10,000 g for 10 min. A 0.1 ml sample of the supernatant is diluted to 10 ml in .02 M Tris buffer pH 7.4. A 30 λ aliquot of the diluted sample is reacted with 0.1 of enzyme substrate reagent (DuPont Reagent Kit). The ATP concentration of sample is calculated by comparing with an ATP standard curve.

Section III. Frozen thin sections

Papillary muscles are frozen in aluminum tongs cooled in liquid nitrogen. The frozen sample is incorporated into a frozen pellet of .3 M perchloric acid. Care is taken to avoid thawing of the muscle during this procedure. Then sections (8 μ) of the muscle and frozen pellet are cut on a refrigerated microtome

(I.E.C., Harris - International Cryostat) at a temperature of -15°C. The frozen ribbon of tissue and perchloric acid is transferred directly from the microtome blade to a cold centrifuge tube. The sample is then allowed to extract for 1/2 hr. in an ice bath. This procedure takes advantage of the lower melting point of the perchloric pellet in the same manner as extraction of frozen powdered muscle. In our hands there is less difficulty with loss of sample than we experience with preparation of a frozen powder.

G-32-631

GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA 30332

OFFICE OF
THE DIRECTOR OF
FINANCIAL AFFAIRS

October 27, 1977

Georgia Heart Association, Inc.
Broadview Plaza - Level C
2581 Piedmont Road, N. E.
Atlanta, Georgia 30324

Gentlemen:

Enclosed in duplicate are Final Financial Reports for the grant-in-aid awards to Drs. Hyland Yu-Liang Chen and Gary L. Anderson at the Georgia Institute of Technology for the period July 1, 1976 through June 30, 1977.

Also enclosed is Georgia Institute of Technology check number 063578 representing unexpended funds from Dr. Chen's grant of $5.79 and $130.12 from Dr. Anderson's grant.

If you have questions or desire any additional information, please let us know.

Sincerely yours,

Evan Crosby
Associate Director of
Financial Affairs

EC/bs
Enclosures as stated

cc: Dr. Hyland Yu-Liang Chen w/c of his report
Dr. Gary L. Anderson w/c of his report
Dr. M. E. Raville w/c of Dr. Chen's report
Dr. J. W. Crenshaw w/c of Dr. Anderson's report
Mr. E. E. Renfro w/c of both reports
Mr. A. H. Becker w/c of both reports
File E-23-622
File G-32-631

Broadview Plaza - Level C
2581 Piedmont Road, N.E.
Atlanta, Georgia 30324

FINANCIAL REPORT FORM

for

(xx G.H.A. RESEARCH GRANT-IN-AID

G-32-631

( ) G.H.A. RESEARCH FELLOWSHIP

TO: Fiscal Officer, Dr. Gene M. Nordby
Vice President for Business & Finance

It will be appreciated if you will provide at your earliest convenience the information requested below on the following project:

TITLE OF PROJECT: Effect Upon "Criticle ATP Level in Heart Muscle

PRINCIPAL INVESTIGATOR: Gary L. Anderson, Ph.D.

AMOUNT OF GRANT:

Principal $ 9,762

Overhead $ 976

TOTAL . . . . . $ 10,738

TO: Georgia Heart Association, Inc.

The following information submitted in accordance with conditions under which the above described grant was made.

EXPENDITURES FROM PRINCIPAL GRANT FROM JULY 1, 1976 TO JUNE 30, 1977.

Category	Amount	Category	Amount
Personnel	$ 6,499.50	Travel	$ 412.76
Retirement Benefits			
~~Equipment~~	$ 128.92	Other: Capital Outlay	$ 1,335.00
Supplies	$ 1,267.34	Overhead	$ 964.36

TOTAL THROUGH JUNE 30 . . . . . $ 10,607.88

*BALANCE OF PRINCIPAL UNEXPENDED AS OF JUNE 30 . . . . . $ 130.12

Comments:

Signed: G. L. Anderson 10/14
Date

Submitted by: Evan Crosby
Associate Dir. of Fin. Affairs
Institution: Georgia Institute of Technology

Date: 10/27/77

* For Research Fellowships, only this question is applicable